INTESTINAL HISTOLOGICAL RESPONSE AFTER A MEAL IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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HISTOLOGIJA CREVA PASTRMKE (*ONCORHYNCHUS MYKISS*)
PRE I POSLE OBROKA

Apstrakt

Peharaste ćelije se nalaze u crevu riba, one sintetišu neutralne i sulfatne mucine i izlučuju sluz, podmazujući nesvareni materijal koji napreduje prema rektumu i štiteći sluzokožu digestivnog trakta. Promene u peharastim ćelijama creva riba posle obroka mogu pokazati odgovor na pojedinačni obrok obzirom na njihovu ulogu u procesu varenja. Cilj ovog istraživanja bio je procena promena histologije creva, pre hranjenja i 6h i 12 h posle obroka. Izgleda da 6h posle obroka kada je vrhunac sinteze proteina kod pastrmke, proces varenja je u toku, a peharaste ćelije izlučuju sluz pa se njihova veličina smanjuje. Slične dimenzije ovih ćelija pre obroka i 12 h posle ishrane potvrđuju da pastrmku treba hraniti 2 puta dnevno, a da drugo hranjenje treba obaviti 6 sati posle prvog. Ovo istraživanje daje više uvida u upravljanje ishranom u procesu uzgoja pastrmki.

Kjlučne reči: *peharaste ćelije, histologija creva, pastrmka*
Keywords: goblet cells, intestine histology, trout

INTRODUCTION

Goblet cells are common components of the post-gastric mucosa in fish and they are the dominant mocous cells in the intestine (Buke, 1971; Groman, 1982). Their nucleus can be found to the bottom part of the cell while mucus fills the upper part and is discharged through an apical pore. Goblet cells can synthesize neutral and sulphate mucins, and sialomucins containing sialic acid (Khojasteh, 2012). The mucus secreted by goblet cells lubricates undigested materials for onward progression into the rectum and protects the mucosa of the digestive tract. Intestinal mucins may have a possible role in osmoregulation (Khojasteh,

2012). Post-prandial changes in the goblet cells in fish intestine may be a response to a single meal since they have a potential role in the digestion process. Measurement of protein synthesis rates in fish can be used as a tool to compare diets and explain some nutritional effects. In fish, generally protein synthesis rates are higher between 4 and 12 hours after a meal. The aim of this study was to assess the changes of intestine histology following a single meal, before feeding and at 6 hours (6h) and 12 hours (12h) after feeding.

MATERIALS AND METHODS

120 juvenile rainbow trout individuals mixed sex (*Oncorhynchus mykiss*), weighing approximately 44.98±1.08 g, were stocked in three 250 l freshwater tanks. Fish were fed *ad libitum* by hand two times daily at 09:00 and 15:00 for 5 weeks a commercial diet. At the end of the experiment, fish were fasted for 24h. Three fish, one fish from each tank were removed, sacrificed by anaesthesia (diluted 1:1 phenoxyethanol in ethanol) and a blow to the head and used as the prefeeding group for measurements of the intestine histology. The remaining trout were fed normally and a group of 6 trout was selected at random, sacrificed and removed at 6h and 12h after feeding, respectively. For light microscopy, rainbow trout midgut samples were first fixed in 10% buffered formalin for 24 h at 4ºC and then immediately dehydrated in graded series of ethanol, immersed in xylol, and embedded in paraffin wax. Sections of 5-7 μm were mounted. After they had been deparaffinized, the sections were rehydrated, stained with Alcian blue, and mounted with Cristal/Mount. Digital images of random cross sections of the midgut were selected in order to measure the mean number of the goblet cells per μm of intestinal fold and the size of the goblet cells. All measurements were made by the ZEN microscope software of ZEISS.

RESULTS AND DISCUSSION

The mean number of the goblet cells per μm of intestinal fold before feeding and at 6h and 12h after feeding was similar (0.07 ± 0.004, 0.06 ± 0.004 and 0.06 ± 0.003, respectively) ($p > 0.05$, Table 1). Regarding the size of the goblet cells the results were 174.69 ± 15.97 μm^2 before feeding, and 108.82 ± 6.97 μm^2 and 159.95 ± 9.99 μm^2 at 6 h and 12 h after feeding respectively ($p < 0.05$, table 2). Smaller goblet cells appeared at 6 h after feeding while the intestine 12 h after feeding and before feeding had similar size of goblet cells ($p > 0.05$).

Table 1. Goblet cells mean number per μm of intestinal fold before feeding, 6 hours and 12 hours after feeding.

Time	Mean number of goblet cells
Before feeding	$0.07^a \pm 0.004$ (12)
6 hours after feeding	$0.06^a \pm 0.004$ (17)
12 hours after feeding	$0.06^a \pm 0.003$ (10)

Data are presented as means ± S.E. The number of intestinal folds is given within parenthesis. Means in a column followed by the same superscript are not significantly different ($p > 0.05$).

Table 2. Goblet cells size (μm^2) before feeding, 6 hours and 12 hours after feeding.

Time	Mean size (μm^2) of goblet cells
Before feeding	$174.69^a \pm 15.97$ (50)
6 hours after feeding	$108.82^b \pm 6.97$ (50)
12 hours after feeding	$159.95^a \pm 9.99$ (50)

Data are presented as means ± S.E. The total numbers of goblet cells are given within parenthesis. Means in a column followed by the same superscript are not significantly different ($p > 0.05$).

Goblet cells are very important for the nutrition of the fish and its health. According to van den Ingh et al. (1991) an increased amount of goblet cells in the epithelium of the intestine could be a sign of enteritis. Such damage is usually related to distal parts of the gastrointestinal tract and characterized also by goblet cell hypertrophy and hyperplasia. Bozic *et al.,* (2001) observed that starvation induced an increase in the number of intestinal goblet cells in carp. The mucus secreted by goblet cells lubricates undigested materials for onward progression into the rectum. Our result shows that the size of the goblet cells is decreased 6 h after feeding a single meal and gained their original size 12 h after feeding. It seems that at 6 h after feeding which is the peak of the protein synthesis rates in trout, the digestion process is on progress and goblet cells secrete their mucous by decreasing their size. 12 h after feeding the protein synthesis rates are decreasing and the digestion process is towards the end, thus the production of mucous by the goblet cells is not needed. The similar size of the goblet cells before feeding and at 12 h after feeding confirms that rainbow trout should be fed twice per day and the second feeding should take place 6 h after the first feeding. This study gives more insight into the feeding management of trout aquaculture.

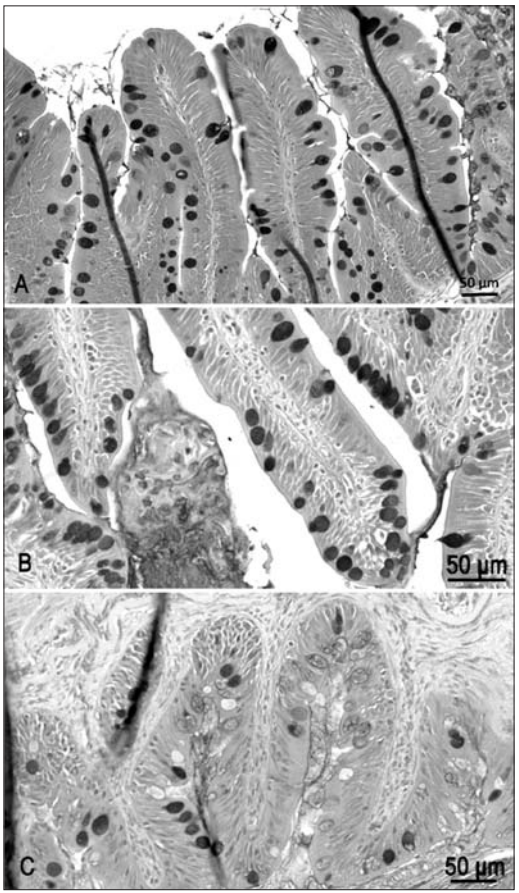

Figure 1. Rainbow trout intestine A: Before feeding. Goblet cells are blue. B: 6 h after feeding. The goblet cells are smaller in size than the ones before feeding. C: 12 h after feeding. The goblet cells have similar size with the ones before feeding.

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